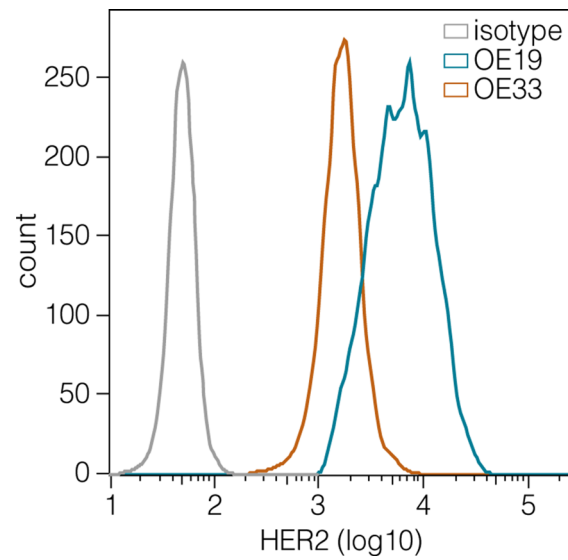
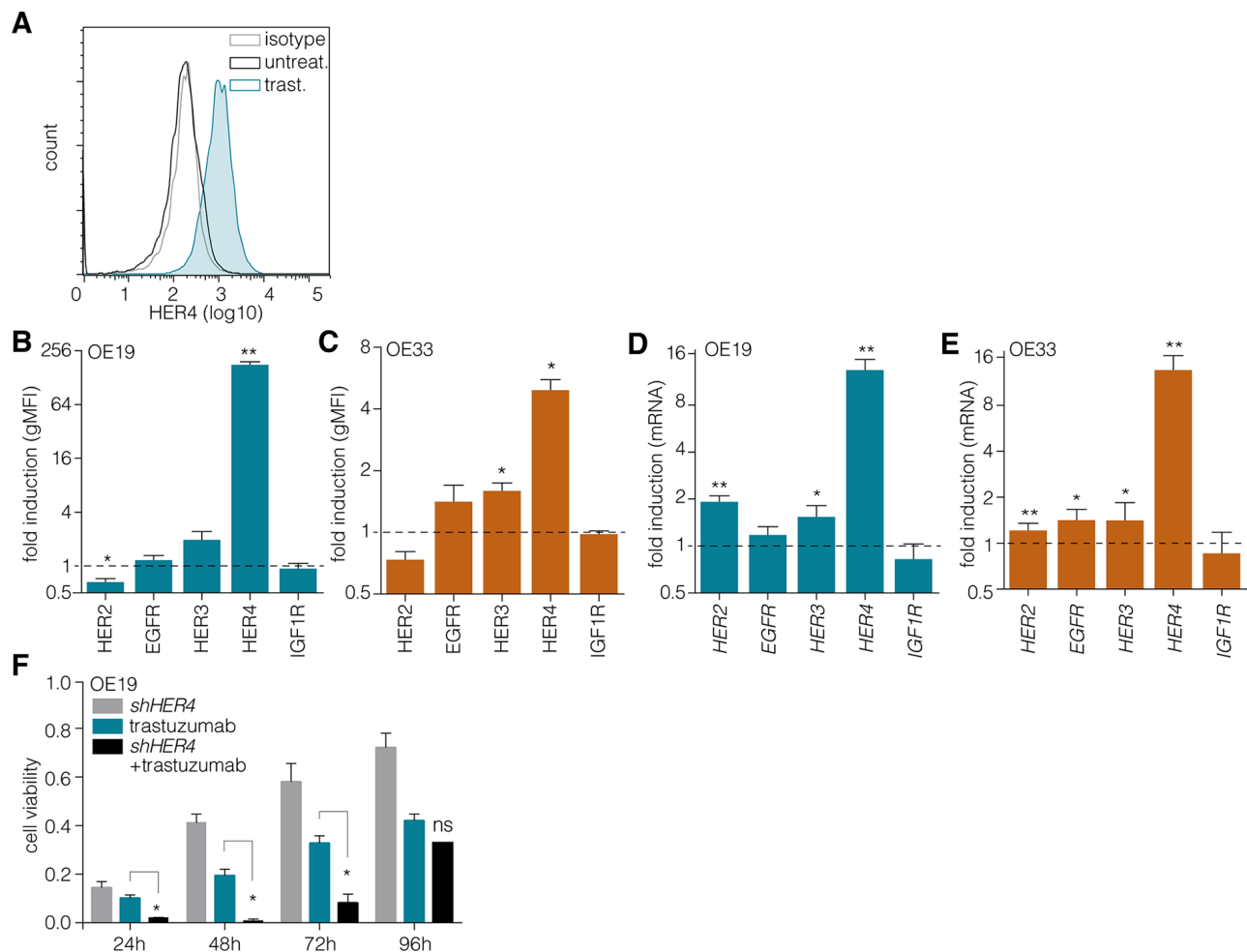


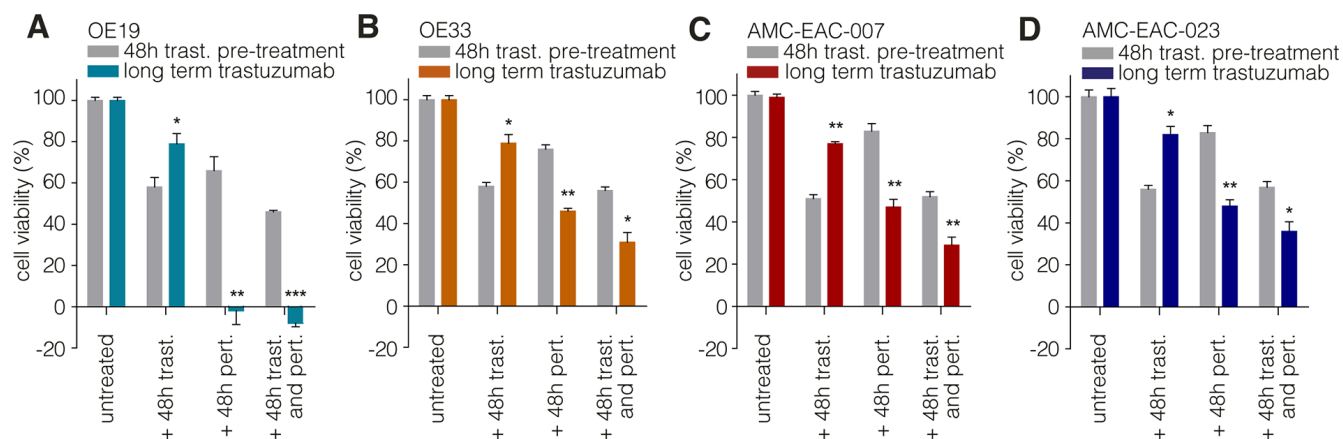
SUPPLEMENTARY FIGURES AND TABLE



Supplementary Figure S1: HER2 cell surface expression. Representative flow cytometry staining for HER2 on EAC cell line OE19 and OE33.



Supplementary Figure S2: HER4 is involved in short-term trastuzumab resistance. **A.** Representative flow cytometry staining for HER4 on OE19 cells after 48 h PBS control or trastuzumab treatment (1 μ g/ml). **B, C.** Indicated HER levels on OE19 and OE33 cells upon 1 μ g/ml trastuzumab treatment (48h) were determined using flow cytometry for indicated proteins. Data were calculated using the gMFI, and values were normalized to the untreated condition (set to 1, not shown in graph). **D, E.** Indicated HER levels were determined by qPCR. Relative expression values were calculated and normalized to GAPDH according to the comparative threshold cycle (Cp) method and normalized to untreated cells. **F.** Cell viability of OE19 cells with doxycycline-induced knockdown of HER4 (shHER4, knockdown efficiency 97%) with or without 1 μ g/ml trastuzumab were measured using the Cell Titer Blue assay. Data show the mean, relative to control (set to 1). Error bars represent the s.e.m. of three independent experiments. (*P-value <0.05, **P-value <0.01).



Supplementary Figure S3: Targeting EGFR in trastuzumab resistant cells. The short term treated cells (grey bars) were first treated with trastuzumab for 48h, followed by a 48h incubation with either panitumumab alone or a combination of trastuzumab and panitumumab. As a control, cells were first cultured short-term (48h) with trastuzumab followed by an additional trastuzumab treatment period of 48h. Trastuzumab indicated on x-axis means that treatment was continued during the experiment along with the other indicated treatments. Cell viability was measured using CTB assay prior to treatment (input) and after treatment. Values are corrected for input and normalized to the control treated condition. Data show the mean \pm s.e.m., $n \geq 3$. (* = P-value <0.05, ** = P-value <0.01, *** = P-value <0.001).

Supplementary Table S1: Primer sequences for shRNA cloning and PCR

transcript	sequence
<i>shHER4</i> Fw	CCGGCCGGCCTGTGGCTATTAAGATTCTTCTCGAGAAGAATCTTAATAGCCACAGGTTTTT
<i>shHER4</i> Rv	AATTAAAAACCTGTGGCTATTAAGATTCTTCTCGAGAAGAATCTTAATAGCCACAGGCCGG
<i>ADAM10</i> sh1	GCAGGTTCTATCTGTGAGAAA
<i>ADAM10</i> sh2	GCTGTGCAGATCATTCAAGTAT
<i>ADAM17</i> sh1	CCTGGTTACAACCTCATGAATT
<i>ADAM17</i> sh2	CCTATGTCGATGCTGAACAAA
<i>EGFR</i> Fw	GTGATCCAAGCTGTCCCAAT
<i>EGFR</i> Rv	ACTGGTTGTGGCAGCAGTC
<i>HER3</i> Fw	TGGGGAACCTTGAGATTGTG
<i>HER3</i> Rv	GAGGTTGGGCAATGGTAGAG
<i>HER4</i> Fw	CATGGCCTTCCAACCTGACT
<i>HER4</i> Rv	TCTGGCAATGATTTTCTGTGG
<i>IGF1R</i> Fw	GTCGAAGAATCGCATCATCA
<i>IGF1R</i> Rv	GCATCCTGCCCATCATACTC